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1 **Abstract**

2 Terbutaline is a prohibited drug except for athletes with a
3 therapeutic use exemption certificate; terbutaline's effects on
4 endurance performance are relatively unknown.

5 Purpose: To investigate the effects of two therapeutic (2mg; 4mg)
6 inhaled doses of terbutaline on 3km running time-trial
7 performance.

8 Methods: Eight males (24.3±2.4yrs; 77.6±8kg; 179.5±4.3cm)
9 and eight females (22.4±3yrs; 58.6±6kg; 163±9.2cm) free from
10 respiratory disease and illness provided written informed
11 consent. Participants completed 3 km running time-trials on a
12 non-motorised treadmill on three separate occasions following
13 placebo, 2 mg or 4 mg inhaled terbutaline, in a single-blind,
14 repeated-measures design. Urine samples (15mins post-exercise)
15 were analysed for terbutaline concentration. Data were analysed
16 using one-way repeated measures ANOVA, significance was set
17 at $p<0.05$ for all analyses.

18 Results: No differences were observed for completion times
19 (1103±201; 1106±195; 1098±165s; $P=0.913$) for the placebo
20 trial, the 2mg inhaled trial and the 4mg inhaled trial, respectively.
21 Lactate values were higher ($P=0.02$) following 4mg terbutaline
22 ($10.7\pm2.3\text{mmol}\cdot\text{L}^{-1}$) vs. placebo ($8.9\pm1.8\text{mmol}\cdot\text{L}^{-1}$). FEV₁
23 values were greater following inhalation of 2mg (5.08 ± 0.2 ;
24 $P=0.01$) and 4mg terbutaline (5.07 ± 0.2 ; $P=0.02$) compared to
25 placebo ($4.83\pm0.5\text{L}$) post-inhalation. Urinary terbutaline
26 concentrations were mean ($306\pm288\text{ng}\cdot\text{mL}^{-1}$; $435\pm410\text{ng}\cdot\text{mL}^{-1}$;
27 $P=0.2$) and peak ($956\text{ng}\cdot\text{mL}^{-1}$; $1244\text{ng}\cdot\text{mL}^{-1}$) following 2mg and
28 4mg inhaled terbutaline, respectively. No differences were
29 observed between the male and female participants.

30 Conclusions: Therapeutic dosing of terbutaline does not lead to
31 an improvement in 3 km running performance despite
32 significantly increased FEV₁. Our findings suggest that athletes
33 using inhaled terbutaline at high therapeutic doses to treat
34 asthma will not gain an ergogenic advantage during 3 km
35 running performance.

36

37 **Introduction**

38 Short-acting β_2 -agonists are used therapeutically by athletes with
39 asthma related conditions to prevent and/or reverse the
40 bronchoconstriction of the airways, leading to restoration of
41 airway function.¹⁻⁵ The majority of athletes treat symptoms of
42 exercise-induced bronchoconstriction (EIB) through the use of
43 salbutamol, making it the most commonly used inhaled β_2 -
44 agonist in these individuals.⁴ However other β_2 -agonists, such as
45 terbutaline, are available which is a suitable alternative to
46 salbutamol, should an athlete not respond appropriately to
47 salbutamol treatment.⁶⁻¹⁰ Athletes that are subject to World Anti-

48 Doping Agency (WADA) regulations, who require alternative
49 β_2 -agonist therapy can apply for a therapeutic use exemption
50 certificate (TUE) in order to use inhaled terbutaline.¹¹

51 The prohibited status of terbutaline is due, in part, to the inability
52 to distinguish between therapeutic inhaled and therapeutic oral
53 doses (with all oral β_2 -agonists being banned under the WADA
54 code), given that an oral dose far exceeds the inhaled dose in
55 terms of the systemic bioavailability when given
56 therapeutically.^{12,13} In some athletes the need for the use of
57 terbutaline is justified, however there are currently no measures
58 in place to prevent an athlete with a legitimate TUE for
59 terbutaline from using the medication at a suprathreshold dose
60 with impunity.¹³

61 The current WADA guidelines monitor the use of the inhaled
62 short-acting β_2 -agonists, salbutamol and formoterol via a urinary
63 threshold limit, above which will present an adverse analytical
64 finding (AAF).¹¹ For salbutamol this limit is 1000 ng·mL⁻¹ with
65 a decision limit of 1200 ng·mL⁻¹ and for formoterol this limit is
66 40 ng·mL⁻¹ with a decision limit of 50 ng·mL⁻¹, with any levels
67 over this presenting an AAF. The current guidelines for use
68 indicate that no more than 1600 μ g salbutamol can be inhaled in
69 a 24 hour period and within this no more than 800 μ g can be
70 inhaled in a 12 hour period, with the equivalent for formoterol
71 being 54 μ g over a 24 hour period.¹¹ If a threshold for terbutaline
72 could be determined, this would enable it to be monitored in
73 much the same way as both salbutamol and formoterol,
74 preventing an athlete with a TUE for terbutaline from potentially
75 using the medication at a suprathreshold dose. Recently
76 Jacobson et al.,¹³ presented the case for establishment of dosing
77 thresholds for terbutaline, these dosing thresholds are extremely
78 important given recent evidence of ergogenic effects of
79 suprathreshold dosages of inhaled terbutaline on sprint and
80 power performance, muscle strength and muscle hypertrophy, as
81 well as inducing muscle phenotype alterations.^{8,9,14,15}

82 The establishment of a urinary threshold for terbutaline has
83 proven to be difficult to attain, recently Dyreborg et al.,¹⁶
84 examined high-dose (4 mg) inhaled versus oral (10 mg)
85 terbutaline, finding that the bioavailability and pharmacokinetics
86 vary distinctly between routes of administration. Peak urinary
87 concentration of 4 mg inhaled terbutaline occurred 2 hours post-
88 inhalation and peak urinary concentration of 10 mg oral
89 terbutaline occurred 6 hours post-ingestion, interestingly there
90 was also no significant difference between urinary levels of
91 inhaled vs oral terbutaline at the 6 hour stage. Similar work was
92 previously performed by Elers et al.,¹² in which inhaled (2 mg)
93 and oral (10 mg) terbutaline were examined, the study found that
94 although there was a significant difference between urine
95 concentrations dependent upon route of administration, no

96 threshold was able to be established due to high variability
97 between individuals. It is therefore important to assess urinary
98 levels of terbutaline for doping control purposes.

99 Evidence exists that the use of terbutaline at a suprathreshold
100 dose has the potential to be ergogenic.^{17,18} These purported
101 effects are due to the fact that short-acting β_2 -agonists (a class of
102 sympathomimetic amines) are able to activate the β_2 adrenergic
103 receptors within the body, which are mainly present on bronchial
104 smooth muscle.¹⁹⁻²¹ Activation of the β_2 adrenergic receptors
105 reverses the constriction of bronchial smooth muscle during
106 bronchoconstriction. These same β_2 receptors are also present on
107 cardiac smooth muscle and skeletal muscle.^{21,22} Adrenergic
108 activation of skeletal muscle has the potential to improve
109 musculoskeletal function and thus has the potential to be
110 ergogenic during exercise performance.²³ Recent investigations
111 suggest an acute suprathreshold inhaled dose (15 mg) of
112 terbutaline may have ergogenic action in sprint cycling
113 performance.^{8,9,14,18} This 15 mg dose is approximately eight
114 times the recommended therapeutic dose for inhaled terbutaline
115 and in athletes with a TUE this would not be permitted according
116 to the WADA code,¹¹ however current regulations would not be
117 able to accurately detect this misuse of terbutaline, due to a lack
118 of urinary thresholds with which to monitor terbutaline use.
119 Given the ergogenic potential of suprathreshold inhaled
120 terbutaline, it remains to be determined whether athletes using
121 terbutaline therapeutically to treat asthma symptoms could also
122 experience an ergogenic effect, traditionally the therapeutic dose
123 of inhaled terbutaline is between 1-2 mg, however studies have
124 shown therapeutic use as high as 4 mg.^{10,16}

125 The aim of the present study was to examine the potential
126 ergogenic action of 2 mg and 4 mg inhaled terbutaline on
127 exercise performance during a 3 km running time-trial and to
128 measure urinary thresholds of terbutaline post-exercise
129 performance.

130

131 **Methods**

132 Following ethical approval from the Liverpool John Moores
133 University research ethics committee (Ethics No. P11SPS044),
134 eight males (age: 24.3 ± 2.4 years; weight: 77.6 ± 8 kg; height:
135 179.5 ± 4.3 cm) and eight females (age: 22.4 ± 3 years; weight:
136 58.6 ± 6 kg; height: 163 ± 9.2 cm) volunteered to participate in
137 the study, providing their written informed consent. All
138 participants were in good health, non-smokers and took part in
139 sport and exercise activities for at least 3 hours per week. No
140 participant had previously been diagnosed with asthma and/or
141 EIB, all participants were free from chest infection for at least
142 two weeks prior to testing. Participants presented with a negative

143 eucapnic voluntary hyperpnoea (EVH) challenge.^{24,25} No
144 participants competed at a level where they were subject to
145 regular anti-doping tests. Participants were informed about the
146 nature and the risks of the experimental procedures before
147 providing written informed consent.

148

149 *3 km Time-Trial*

150 The 3 km time-trials were conducted on a non-motorised curved
151 treadmill (Woodway Curve, Woodway, USA). Participants were
152 familiarised to running on a non-motorised treadmill prior to
153 initiating their recorded 3 km time-trials. Familiarisation runs
154 took place on at least two occasions and participants progressed
155 to the recorded 3 km time-trials only once they felt comfortable
156 pacing themselves on the non-motorised curved treadmill over a
157 3 km distance (Figure 1).

158 Each participant was required to perform a 3km time-trial on
159 three occasions in a randomised, single blind, repeated measures
160 design with a minimum of 7 days between trials. Participants
161 were instructed to follow the same 24-hour dietary intake prior
162 to each trial and were instructed to abstain from caffeine for 6
163 hours before attending. Prior to completing the 3 km time-trial
164 participants completed baseline maximal flow-volume
165 manoeuvre in accordance with ERS/ATS criteria.²⁴ Following
166 baseline spirometry participants inhaled either eight inhalations
167 of non-active inhalant (placebo), four inhalations of non-active
168 inhalant plus four inhalations of 0.5 mg terbutaline (2 mg) or
169 eight inhalations of 0.5 mg terbutaline (4 mg). Participants
170 received the inhaled terbutaline via turbuhaler (Bricanyl,
171 Turbuhaler, AstraZeneca, Canada), participants were advised to
172 inhale at a steady flow-rate for 2 seconds until full inhalation and
173 to hold each inhalation for 10 seconds, a minimum of 1 minute
174 was required between each subsequent inhalation. Ten minutes
175 post-inhalation spirometry was repeated, before the completion
176 of a standardised warm-up (5 minutes on a motorized treadmill
177 at 10 kph). The 3km time-trials were performed under controlled
178 laboratory conditions of 18°C, 20.9% O₂ and 40% humidity.

179 During the time-trial participants wore a heart rate monitor
180 (Polar RS400; Polar Electro Oy, Kempele, Finland) and face-
181 mask connected to a breath-by-breath gas analyser (Oxycon Pro,
182 Jaeger, Wurzburg, Germany). Every 0.5 km the following
183 variables were measured: time (s), heart rate (HR), oxygen
184 consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute
185 ventilation (\dot{V}_E), respiratory exchange ratio (RER) and rating of
186 perceived exertion (RPE).²⁶ Two minutes following the
187 completion of the 3 km time-trial a finger-tip capillary blood
188 sample was collected to measure blood lactate concentration

189 (Lactate Pro, Arkray KDK, Japan) followed by spirometry and
190 collection of a post-exercise urine sample (Figure 1).

191 During the 3 km time-trial participants were only given feedback
192 on the distance they had covered. They were blind to all other
193 feedback such as time and HR. Participants were encouraged to
194 complete the time-trial as fast as possible, a-priori power
195 calculations for the 3 km running time-trial predicted that for an
196 expected completion time of 1100 seconds, with a standard
197 deviation of (14%) 154 seconds, a sample size of 8 would be
198 sufficient to significantly ($P < 0.05$) predict a 2.5% 27 second
199 change in performance with 80% power.

200 *Urinalysis*

201 Collected urine-samples were measured for pH and osmolality
202 before 30 ml of each sample was distributed into a Nalgene
203 bottle (Thermo Fisher Scientific, Leicestershire, UK) prior to
204 freezing the sample at -80°C until urinalysis. All urinalysis was
205 performed at HFL Sport Science (Fordham, United Kingdom),
206 an independent drug surveillance laboratory and former WADA-
207 accredited laboratory. All samples were packaged in dry ice
208 during transportation to prevent thawing. The laboratory used a
209 validated proprietary analytical method. In brief, urine samples
210 were thawed, centrifuged and subaliquotted prior to addition of
211 a deuterated internal standard (Terbutaline D_3 ; CDN Isotopes via
212 QMX Laboratories Ltd, Thaxted, UK). Following overnight
213 enzymatic hydrolysis with β glucuronidase from *E. Coli* (type
214 1X-A; Sigma Aldrich, Dorset, UK), sample clean-up was
215 performed using solid phase extraction (Strata XC 30 mg 96-
216 well plate; Phenomenex, Macclesfield, UK). After elution,
217 samples were evaporated to dryness, reconstituted and analysed
218 using an AB Sciex 4000 QTrap mass spectrometer (AB Sciex,
219 Warrington, UK), with a Waters Acquity UPLC system (Waters
220 Ltd, Elstree, UK). Chromatographic separation was achieved
221 using a Waters Acquity HSS T3 Column (2.1 x 100 mm, particle
222 size $1.8\ \mu\text{m}$) and gradient solvent programme using methanol
223 and water, both containing 10 mM ammonium formate.

224
225 Sample concentrations were measured using a calibration line
226 containing Terbutaline at different concentrations (10 to 3000
227 $\text{ng}\cdot\text{ml}^{-1}$) which were extracted and analysed in the same batch.
228 Quality control samples were tested along with samples to
229 confirm assay performance.

230 *Sample Correction*

231 All urine concentrations of terbutaline were corrected to a urine
232 specific gravity of 1.02 prior to analysis using the following
233 equation¹²:

234
235

236 Corrected urine concentration = terbutaline urine concentration
237 x (0.02/(urine specific gravity -1)).
238

239 *Statistical Analysis*

240 Statistical analysis incorporated one-way repeated measures
241 analysis of variance (ANOVA) to compare between trial
242 conditions during time-trial performance and two-way ANOVA
243 to compare spirometry measurements between conditions at
244 different time-points, a Bonferroni correction was applied to
245 correct for multiple comparisons. Significance was set at $P <$
246 0.05 for all analyses. All data were reported as mean (\pm SD)
247 unless otherwise stated. Statistical analysis was performed using
248 the statistical package for the social sciences (SPSS v21.0, IBM,
249 New York).

250

251 **Results**

252 Sixteen participants successfully completed all trials, participant
253 demographics and lung function screening values are shown in
254 Table 1. No adverse side-effects were reported by any of the
255 participants during the study.

256

257 There were no significant differences in completion time
258 between trials either within the combined group (1103 ± 194 s;
259 1106 ± 118 s; 1098 ± 160 s; $P = 0.9$) or when groups were split
260 according to gender: female (1249 ± 149.3 s; 1257 ± 112 s; 1215
261 ± 96 s; $P = 0.37$) and male (956 ± 102 s; 955 ± 113 s; 982 ± 122
262 s; $P = 0.28$) for PLA, 2 mg and 4 mg trials respectively (Figure
263 2).

264 Post time trial blood lactate was greater following 4 mg inhaled
265 terbutaline (10.7 ± 2.3 mmol·L⁻¹) when compared to the placebo
266 trial (8.9 ± 1.8 mmol·L⁻¹; $P = 0.02$; Figure 3). There were no
267 differences in gas exchange variables for $\dot{V}O_2$ (49.1 ± 7.7 ; 49.3
268 ± 5.2 ; 48.9 ± 5.1) $\dot{V}CO_2$ (50.3 ± 5.9 ; 52.5 ± 5.5 ; 52.1 ± 4.5) or
269 RER (1.08 ± 0.1 ; 1.09 ± 0.05 ; 1.12 ± 0.07), for placebo, 2 mg
270 inhaled and 4 mg inhaled terbutaline, respectively.

271 Exercising heart rate (HR) did not differ ($P=0.95$) between trial
272 conditions, ratings of perceived exertion (RPE) values did not
273 differ between trials at any time-point during performance
274 (Figure 4).

275 There was a significant difference in FEV₁ between trial
276 conditions post-inhalation of 2 mg and 4 mg terbutaline (Table
277 2). There were no differences between FEV₁ values in the
278 placebo trial following terbutaline administration or following
279 time-trial completion, there was no difference in baseline lung
280 function values between conditions. There was a significant

281 difference in post inhalation FEV₁ values compared to placebo
282 (P=0.007; P=0.003) for both 2 mg and 4 mg inhalation trials,
283 respectively (Table 2; Figure 5). Interestingly, the difference in
284 FEV₁ post time-trial between conditions was not significant
285 (P=0.06) (Figure 5), possibly due to a slightly raised FEV₁
286 following exercise in the placebo trial.

287 There was no significant difference (P=0.195) in urine
288 concentration between either the 2 mg inhalation or the 4 mg
289 inhalation post time-trial in males or females with mean \pm SD
290 for the pooled groups (306 \pm 288 ng·mL⁻¹; 435 \pm 410 ng·mL⁻¹)
291 and the peak values (956 ng·mL⁻¹; 1244 ng·mL⁻¹) for 2 mg
292 inhaled terbutaline and 4 mg inhaled terbutaline, respectively
293 (Figure 6).

294

295 **Discussion**

296 This study demonstrates that inhaled terbutaline (up to 4 mg)
297 does not lead to improved 3 km running time-trial performance
298 in recreationally active individuals. This is despite an observed
299 small improvement in FEV₁ and an increase in post-exercise
300 lactate (4 mg terbutaline only) when compared to placebo.

301 Our study is in agreement with others that suggest there is no
302 significant effect on endurance performance following a high
303 dose of inhaled terbutaline.^{8,9} Previous work investigating the
304 effects of oral supra-therapeutic doses of terbutaline (8 mg)
305 failed to show an ergogenic effect on endurance performance
306 and maximal sprint cycling performance.⁷ Further experiments
307 performed by Kalsen et al.,⁸ examined the effects of high-dose
308 (15 mg) inhaled terbutaline on 300 kcal cycling time-trial
309 performance, there was no difference in completion times (1054
310 \pm 125 s; 1072 \pm 145 s) for placebo vs 15 mg inhaled terbutaline,
311 respectively. These results are comparable to the present study
312 in which completion times were (1102 \pm 125 s; 1098 \pm 109 s) in
313 the pooled groups for placebo vs 4 mg inhaled terbutaline,
314 respectively. This evidence supports a lack of ergogenic
315 potential for terbutaline in moderate duration (~1100 s)
316 endurance running and cycling performance.

317 Hostrup et al.,⁹ reported that high-dose (15 mg) inhaled
318 terbutaline increased muscle strength, and maximal sprint
319 cycling performance but did not enhance endurance cycling
320 performance. In line with these findings, further examination of
321 this acute dose of 15 mg inhaled terbutaline was performed by
322 Kalsen et al.,¹⁴ investigating the effects on maximal 10s sprint
323 cycling performance, with the finding that the observed increase
324 in power output was also associated with increased levels of
325 plasma lactate. They concluded that for a short period of time,
326 terbutaline can counteract a reduction in ATP in type II muscle

327 fibres, further enhancing maximal sprint potential. The general
328 consensus from Kalsen et al.,¹⁴ and Hostrup et al.,⁹ was that 15
329 mg inhaled terbutaline promotes a shift towards anaerobic
330 carbohydrate metabolism during exercise, which may lead to
331 greater power production in short-term anaerobic activity and
332 greater fatigability over longer duration aerobic activity.^{8,9,14,17,18}

333 Following the 4 mg inhaled terbutaline condition we observed
334 an increase in post-exercise lactate ($10.7 \pm 2.3 \text{ mmol} \cdot \text{L}^{-1}$) when
335 compared to placebo ($8.9 \pm 1.8 \text{ mmol} \cdot \text{L}^{-1}$). This may be, in part,
336 due to enhanced Ca^{2+} release and increased contractile properties
337 of skeletal muscle following terbutaline administration.^{14,17,18}
338 Hostrup et al.,¹⁸ suggest that this enhanced contractility of
339 skeletal muscle leads to elevated glycolytic activity during high-
340 intensity exercise. These findings are in accordance with the
341 findings of Kalsen et al.,⁸ when investigating the effect of high-
342 dose (15 mg) terbutaline on steady state exercise and also 300
343 kcal time-trial cycling performance, where lactate accumulation
344 was higher during steady state exercise and was found to be
345 attributable to higher rates of glycogenolysis and glycolysis,
346 with no concomitant improvement in endurance performance. In
347 association with the findings of Kalsen et al.,⁸ it is possible that
348 the lack of ergogenic effect seen in both our study and the study
349 by Sanchez et al.,⁷ can be explained by an earlier onset of fatigue
350 during endurance performance due to enhanced glycolytic
351 activity induced by terbutaline.^{9,14}

352 The improvements seen in other studies with regard to sprint and
353 power performance, could be due to greater potentiation of
354 adrenergic receptors at very high dosages, according to Baker et
355 al.,²⁷ a combination of selective affinity and intrinsic efficacy
356 (ability to induce a response) dictate the strength of response at
357 a given receptor. A highly selective partial agonist of the β_2 -
358 receptor such as terbutaline, with high intrinsic efficacy, given
359 at a supra-therapeutic dose would have the ability to bind to the
360 β_2 -receptors in many types of tissue, increasing the ergogenic
361 potential of the drug.²⁷ This could be one factor that could
362 support the ergogenic effects found in those studies examining
363 15 mg inhaled terbutaline for strength and power
364 performance.^{9,14,17,18} With this in mind, the distribution of the
365 high therapeutic dose (4 mg) in the present study, would likely
366 have been lower than that of the 15 mg inhaled dose studies,
367 therefore there could have been a lower potency of the β_2 -agonist.
368 Given that the present study's evidence stems from
369 recreationally active individuals, it is likely that these results are
370 transferrable to highly trained individuals, (i.e. the physiological
371 response would be the same in both groups). Although this is a
372 limitation, ethically, it would not have been possible to perform
373 this study in an elite population, due to the athletes'
374 responsibility to undertake out-of-competition testing.

375 A TUE is needed for the use of inhaled terbutaline during
376 competition, largely due to the inability to distinguish between
377 route of administration and total dose administered.^{12,16,28} In the
378 present study we were able to measure urine concentrations of 2
379 mg and 4 mg doses of terbutaline, interestingly our values for 2
380 mg ($305.5 \pm 288.3 \text{ ng} \cdot \text{mL}^{-1}$) inhaled terbutaline are lower than
381 those found in a previous investigation by Elers et al.,¹² for 2 mg
382 inhaled terbutaline ($472 \pm 324 \text{ ng} \cdot \text{mL}^{-1}$) and our values after 4 mg
383 inhaled terbutaline ($435.4 \pm 409.8 \text{ ng} \cdot \text{mL}^{-1}$) are comparable to the
384 values after 10 mg oral terbutaline in the Elers et al.,¹² study
385 ($402 \pm 663 \text{ ng} \cdot \text{mL}^{-1}$). Interestingly, these values for both varying
386 dosages and alternate routes of administration have very similar
387 mean values, further highlighting the difficulty in distinguishing
388 between therapeutic and supra-therapeutic use of terbutaline.¹²
389 Of note, the timing of the urine sample in the Elers et al.,¹² study
390 was at 4 hours, whereas in the present study urine samples were
391 collected 1-hour post-inhalation. Indeed, serum concentrations
392 of terbutaline reached a peak at the 4-hour stage in the Elers et
393 al.,¹² study, therefore it is possible that inhaled terbutaline may
394 not have reached peak levels in the urine at the 1-hour sample
395 collection in the present study. The 4 mg inhaled dose was
396 previously examined by Dyreborg et al.,¹⁶ with peak
397 concentrations reaching $1954 \text{ ng} \cdot \text{mL}^{-1}$ at the 2 hour stage post-
398 inhalation, the present study found peak concentrations reaching
399 $1244 \text{ ng} \cdot \text{mL}^{-1}$ 1 hour post-inhalation, it would have been
400 beneficial to examine urinary levels of terbutaline at additional
401 timepoints in the present study in order to ascertain time to
402 maximal concentration (T_{max}) of terbutaline. A number of factors
403 contribute to the varying levels of urinary terbutaline, recent
404 work by Kreiberg et al.,²⁹ indicate varying pharmacokinetics of
405 4 mg inhaled terbutaline dependent upon external factors such as
406 exercise performance and also environmental conditions, these
407 differences exist post-correction for urine specific gravity,
408 explanations for such variance include but are not limited to;
409 inhalation technique, exercise intensity and hydration status.

410 In the investigations by Elers et al.,¹² and Dyreborg et al.,¹⁶
411 significant differences were found between oral and inhaled
412 doses, but no cut-off value could be established. If a cut-off value
413 were able to be established then it is possible that inhaled
414 terbutaline would be able to be monitored in much the same way
415 as both salbutamol and formoterol, where an AAF would
416 indicate possible supra-therapeutic inhaled use or oral
417 administration, which have established ergogenic potential in
418 strength and power performance.^{7-9,14,17,18} Further investigation
419 is needed to establish the ergogenic effects of therapeutic inhaled
420 terbutaline on sprint and power performance. Recent findings
421 also highlight that daily use of 4 mg inhaled terbutaline displays
422 repartitioning properties, allowing for reductions in body fat and

423 increases in muscle mass.³⁰ Care is warranted with regard to the
424 use of terbutaline in athletes with a TUE.

425 **Practical Applications**

426 Therapeutic use of terbutaline in athletes with a TUE will not
427 lead to an ergogenic advantage during running-based endurance
428 exercise. Investigations into appropriate monitoring of
429 terbutaline are warranted in order to prevent the potential misuse
430 of terbutaline via suprathreshold dosing.

431 **Conclusions**

432 The findings of the present study suggest that therapeutic doses
433 of inhaled terbutaline (up to 4 mg) do not improve 3 km running
434 time-trial performance. Endurance running athletes using
435 inhaled terbutaline via TUE, as therapy for their asthma, are
436 therefore unlikely to experience an additional ergogenic
437 advantage. Further research is needed investigating the effects of
438 therapeutic inhaled doses of terbutaline during strength and
439 power performance to fully elucidate any ergogenic potential.

440

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581 **Tables**

582

Table 1: Mean (\pm SD) Participant Demographics and Lung Function at Baseline and % Change in Lung Function Post-EVH in Males and Females.

Group	Height (cm)	Weight (kg)	Age (yrs)	Baseline FEV ₁ (L)	% Predicted FEV ₁	Baseline FVC (L)	% Predicted FVC	FEV ₁ /FVC Ratio	Baseline PEF (L)	% Predicted PEF	Post-EVH % Fall in FEV ₁
Males (n=8)	179.5 (4.3)	77.6 (8)	24.3 (2.4)	5.2 (0.2)	114 (4.6)	5.9 (0.6)	110.5 (8.2)	0.83 (0.05)	580.6 (57.9)	96 (10)	5.1 (6.1)
Females (n=8)	163 (9.2)	58.6 (6)	22.4 (3)	3.6 (0.5)	108.9 (13.4)	3.93 (0.5)	105.3 (12)	0.92 (0.03)	439.1 (75.7)	102.8 (17.8)	3.8 (1.6)

FEV₁ - Forced Expiratory Volume in 1 Second; EVH - Eucapnic Voluntary Hyperpnoea; FVC – Forced Vital Capacity; PEF – Peak Expiratory FlowECCS – *European Community for Coal and Steel Reference Values for Predicted Lung Function*

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Table 2: FEV₁ (L) for trial conditions at baseline, post inhaler and post time trial in the pooled group

Time point	Placebo	2 mg	4 mg
Baseline	4.81 \pm 0.55	4.84 \pm 0.54	4.80 \pm 0.55
Post Inhaler	4.83 \pm 0.54	5.08 \pm 0.55*	5.07 \pm 0.48†
Post Time-Trial	4.87 \pm 0.56	5.07 \pm 0.55	5.04 \pm 0.49

Significantly different from placebo * P=0.01 † P=0.02

FEV₁ – Forced Expiratory Volume in 1 Second

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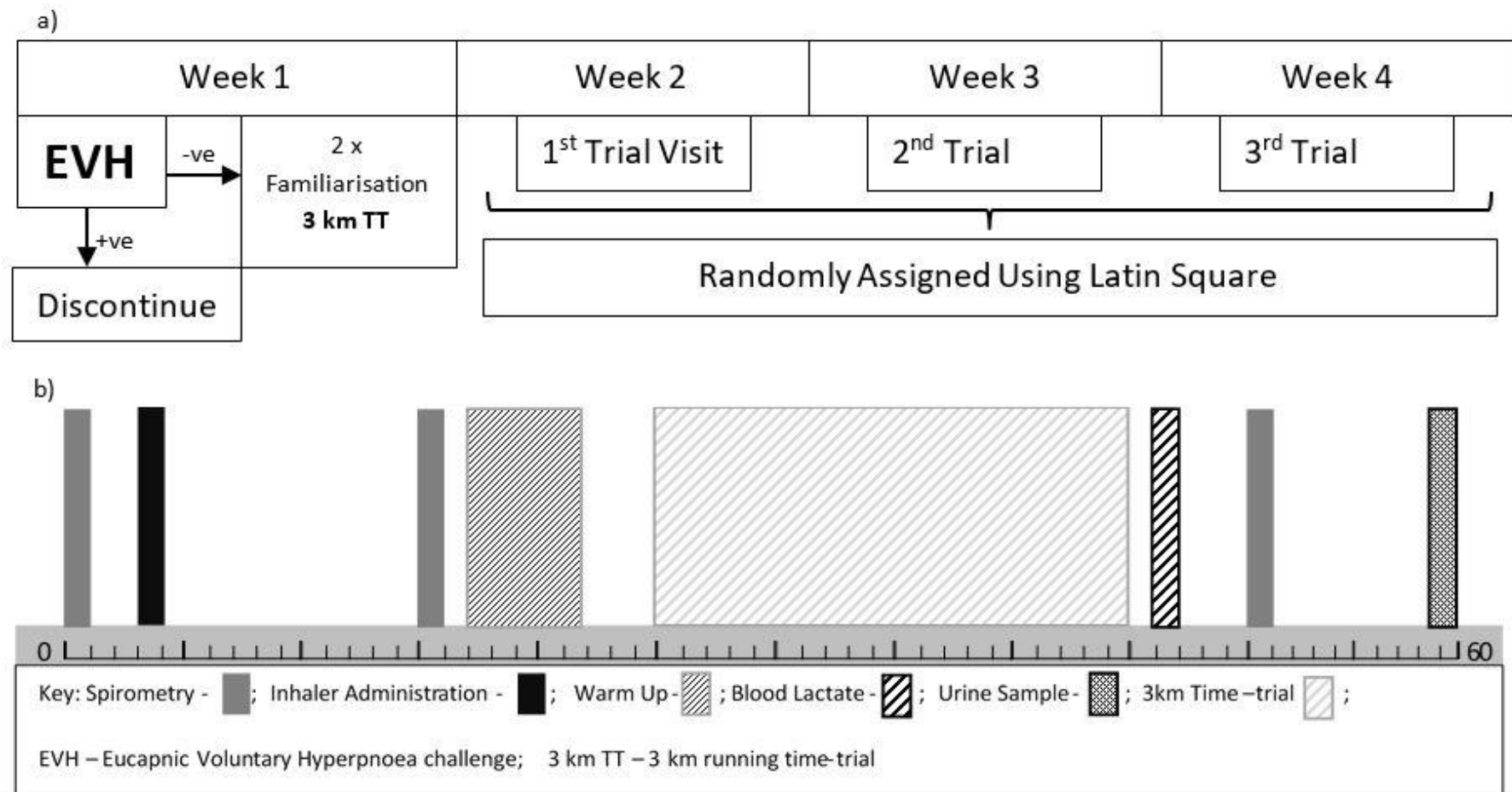
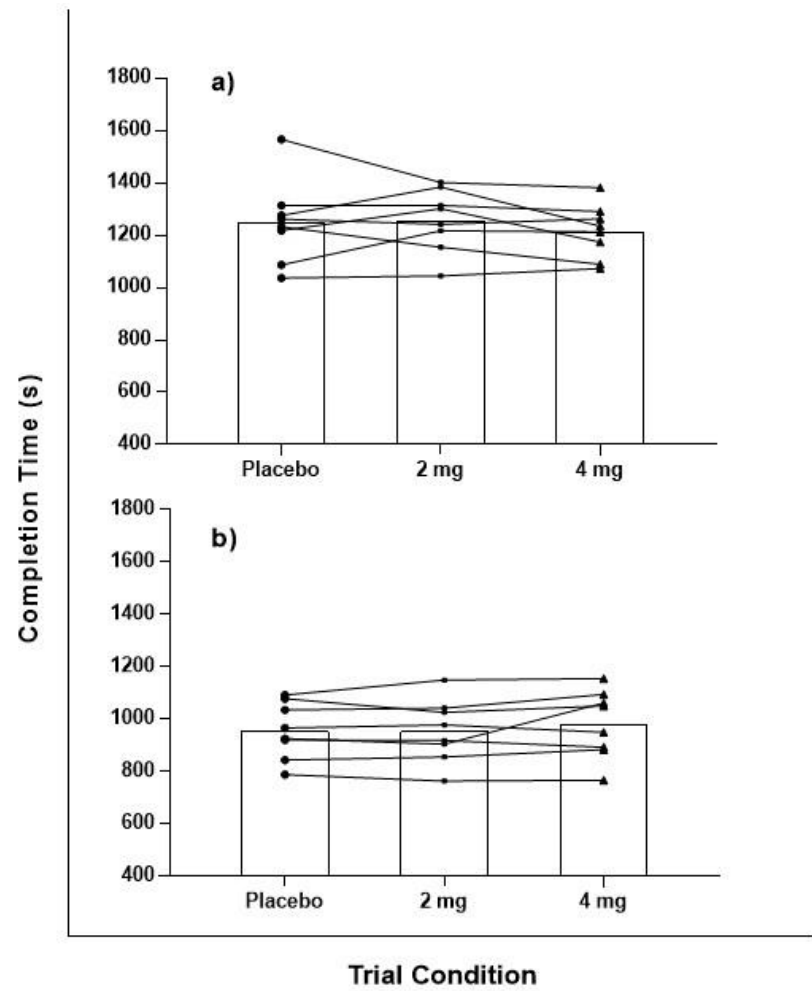


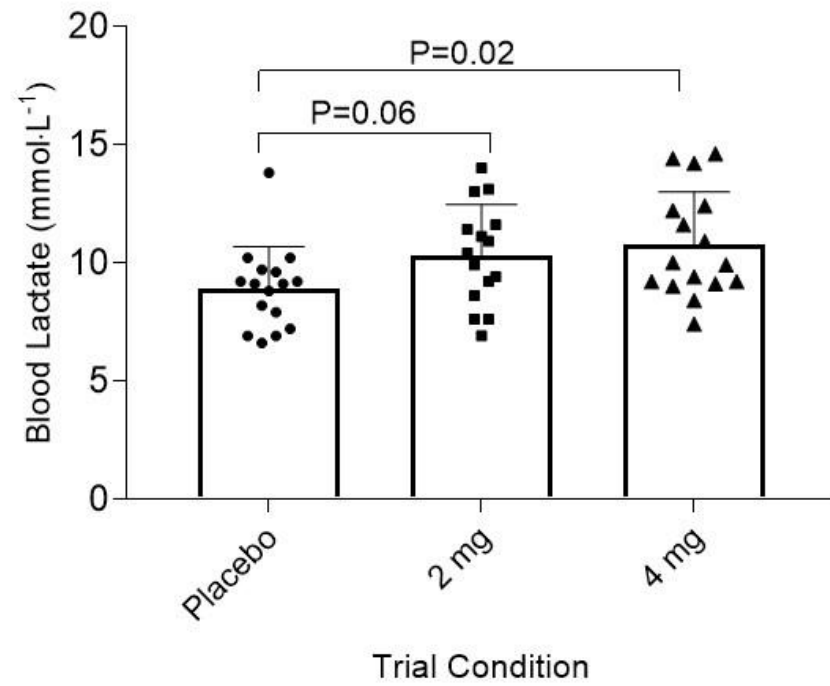
Figure 1: a) Study duration, progression and randomisation protocol b) Schematic diagram of the test procedures during the 60-minute trial visit



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590 **Figure 2:** Mean and individual 3km running time-trial completion times for a) females and b) males following placebo, 2 mg inhaled terbutaline and 4
 591 mg inhaled terbutaline trial conditions.

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595 **Figure 3:** Mean (\pm SD) Lactate values post 3km running time-trial for placebo, 2 mg inhaled terbutaline and 4 mg inhaled terbutaline conditions.

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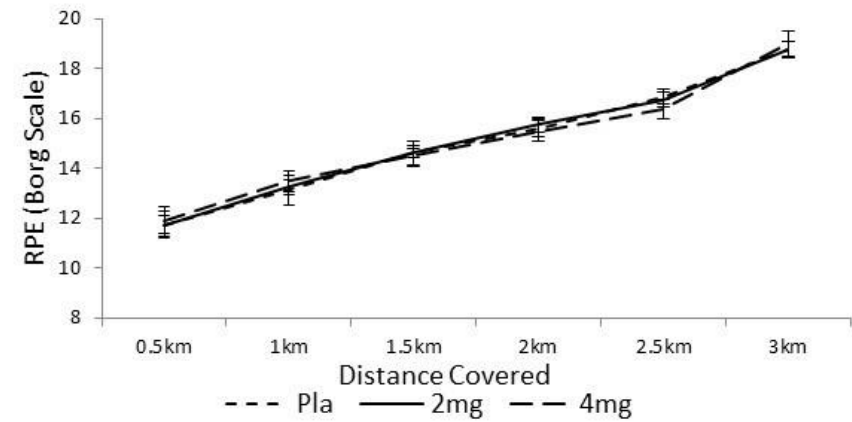
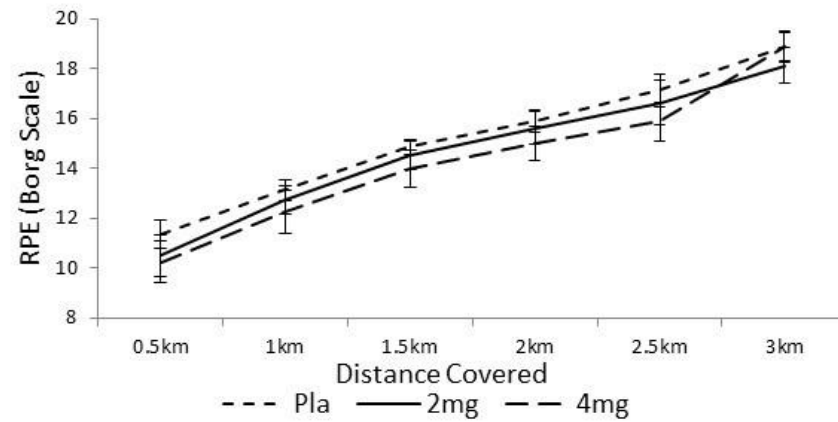
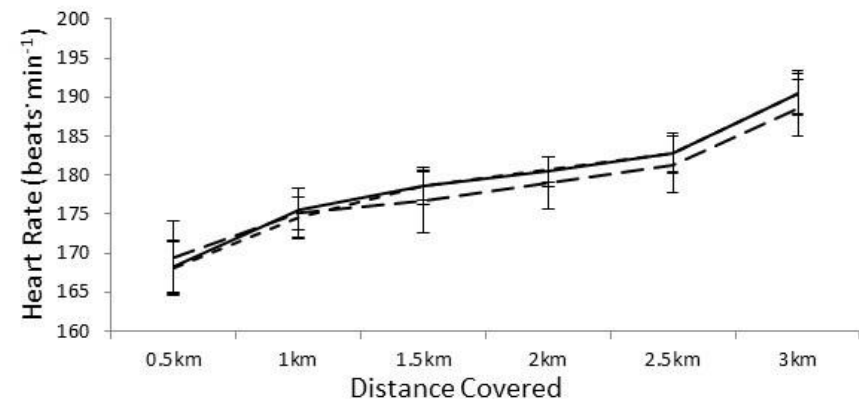
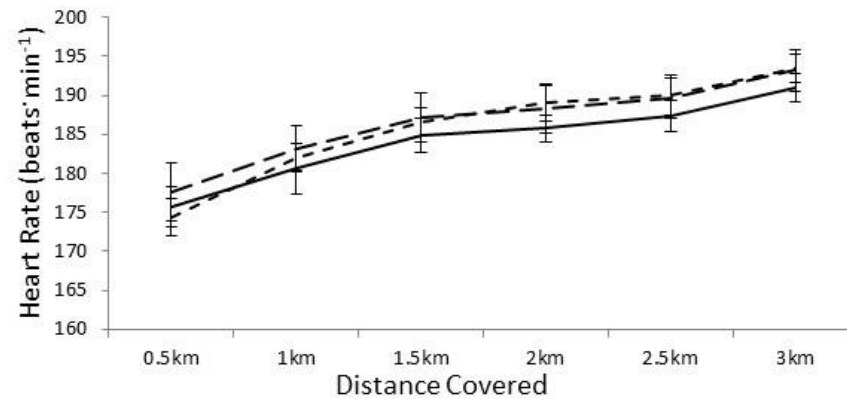
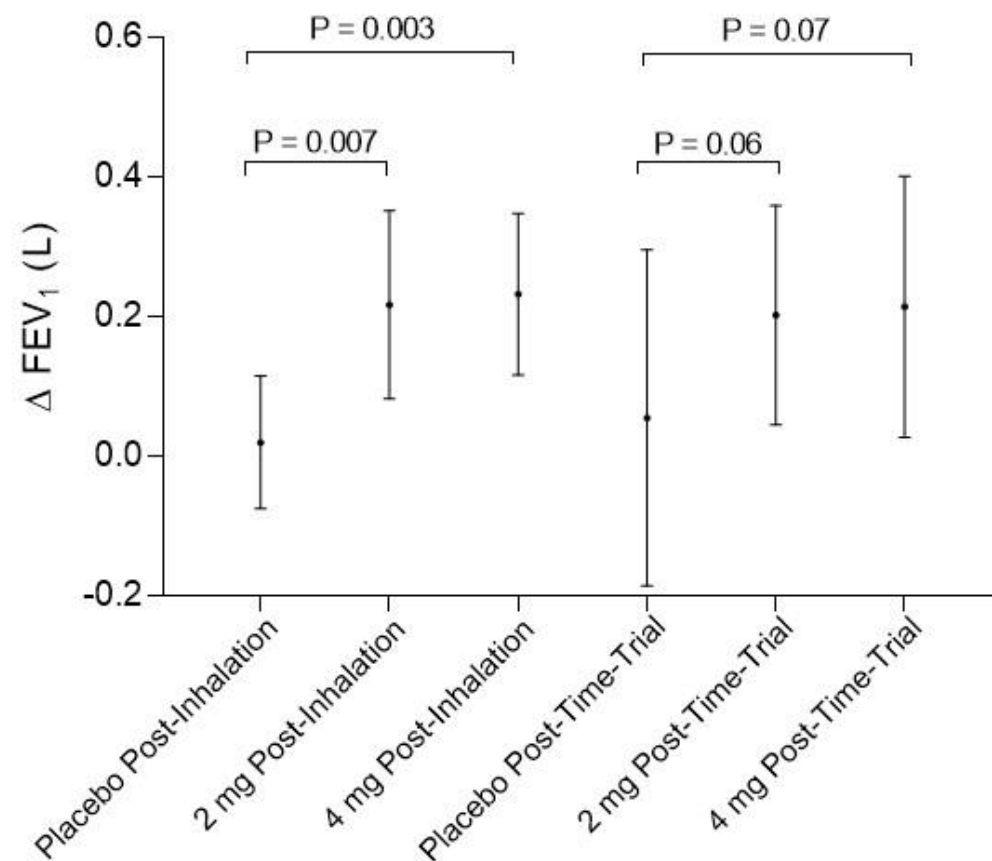


Figure 4: Exercising values (Mean \pm SD) for: Heart Rate (HR) in a) females b) males and rating of perceived exertion (RPE) in c) females d) males during each of the three trial conditions, placebo, 2 mg inhaled terbutaline and 4 mg inhaled terbutaline.

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604 **Figure 5:** Mean (\pm SD) change in FEV₁ from baseline post-inhalation and post-time-trial completion for placebo, 2 mg inhaled terbutaline and 4 mg
 605 inhaled terbutaline.

606 Δ FEV₁ – Change in FEV₁ compared to baseline

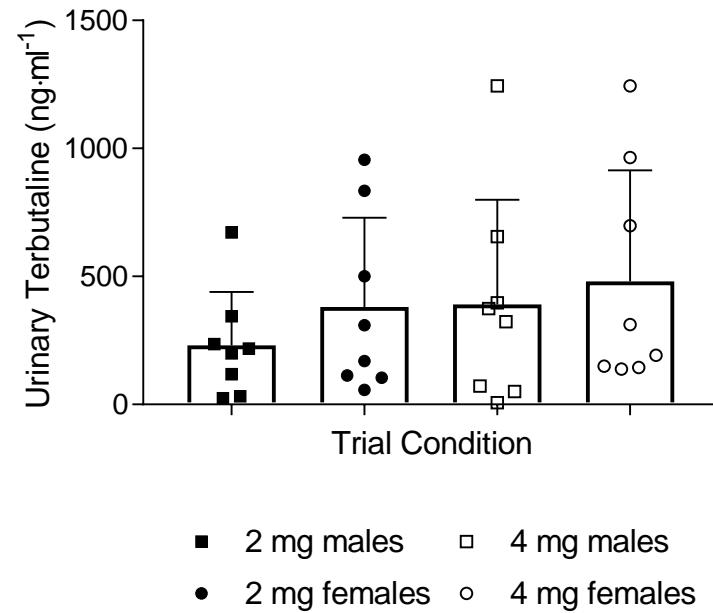


Figure 6: Individual peak and mean (\pm SD) urinary concentrations 1 hour post terbutaline inhalation in the 2 mg inhaled and 4 mg inhaled terbutaline trials in males and females.